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# Conservation Genetics of Exploited Finnish Salmonid Fishes

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To my mother and father

**SWATDIPONG AKARAPONG, CONSERVATION GENETICS OF EXPLOITED FINNISH SALMONID FISHES**

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# Abstract

Genetic diversity is one of the levels of biodiversity that the World Conservation Union (IUCN) has recognized as being important to preserve. This is because genetic diversity is fundamental to the future evolution and to the adaptive flexibility of a species to respond to the inherently dynamic nature of the natural world. Therefore, the key to maintaining biodiversity and healthy ecosystems is to identify, monitor and maintain locally-adapted populations, along with their unique gene pools, upon which future adaptation depends. Thus, conservation genetics deals with the genetic factors that affect extinction risk and the genetic management regimes required to minimize the risk. The conservation of exploited species, such as salmonid fishes, is particularly challenging due to the conflicts between different interest groups.

In this thesis, I conduct a series of conservation genetic studies on primarily Finnish populations of two salmonid fish species (European grayling, *Thymallus thymallus*, and lake-run brown trout, *Salmo trutta*) which are popular recreational game fishes in Finland. The general aim of these studies was to apply and develop population genetic approaches to assist conservation and sustainable harvest of these populations. The approaches applied included: i) the characterization of population genetic structure at national and local scales; ii) the identification of management units and the prioritization of populations for conservation based on evolutionary forces shaping indigenous gene pools; iii) the detection of population declines and the testing of the assumptions underlying these tests; and iv) the evaluation of the contribution of natural populations to a mixed stock fishery.

Based on microsatellite analyses, clear genetic structuring of exploited Finnish grayling and brown trout populations was detected at both national and local scales. Finnish grayling were clustered into three genetically distinct groups, corresponding to northern, Baltic and south-eastern geographic areas of Finland. The genetic differentiation among and within population groups of grayling ranged from moderate to high levels. Such strong genetic structuring combined with low genetic diversity strongly indicates that genetic drift plays a major role in the evolution of grayling populations. Further analyses of European grayling covering the majority of the species' distribution range indicated a strong global footprint of population decline. Using a coalescent approach the beginning of population reduction was dated back to 1 000-10 000 years ago (ca. 200-2 000 generations). Forward simulations demonstrated that the bottleneck footprints measured using the *M* ratio can persist within small populations much longer than previously anticipated in the face of low levels of gene flow. In contrast to the *M* ratio, two alternative methods for genetic bottleneck detection identified recent bottlenecks in six grayling populations that warrant future monitoring. Consistent with the predominant role of random genetic drift, the effective population size ($N_e$) estimates of all grayling populations were very low with the majority of $N_e$ estimates below 50. Taken together, highly structured local populations, limited gene flow and the small $N_e$ of grayling populations indicates that grayling populations are vulnerable to overexploitation and, hence, monitoring and careful management using the precautionary principles is required not only in Finland but throughout Europe.

Population genetic analyses of lake-run brown trout populations in the Inari basin (northernmost Finland) revealed hierarchical population structure where individual populations were clustered into three population groups largely corresponding to different geographic regions of the basin. Similar to my earlier work with European grayling, the genetic differentiation among and within population groups of lake-run brown trout was relatively high. Such strong differentiation indicated that the power to determine the relative contribution of populations in mixed fisheries should be relatively high. Consistent with these expectations, high accuracy and precision in mixed stock analysis (MSA) simulations were observed. Application of MSA to indigenous fish caught in the Inari basin identified altogether twelve populations that contributed significantly to mixed stock fisheries with the Ivalojoki river system being the major contributor (70%) to the total catch. When the contribution of wild trout populations to the fisheries was evaluated regionally, geographically nearby populations were the main contributors to the local catches. MSA also revealed a clear separation between the lower and upper reaches of Ivalojoki river system – in contrast to lower reaches of the Ivalojoki river that contributed considerably to the catch, populations from the upper reaches of the Ivalojoki river system (>140 km from the river mouth) did not contribute significantly to the fishery. This could be related to the available habitat size but also associated with a resident type life history and increased cost of migration.

The studies in my thesis highlight the importance of dense sampling and wide population coverage at the scale being studied and also demonstrate the importance of critical evaluation of the underlying assumptions of the population genetic models and methods used. These results have important implications for conservation and sustainable fisheries management of Finnish populations of European grayling and brown trout in the Inari basin.

# Contents

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

**I** Swatdipong A, Vasemägi A, Koskinen M, Piironen J and Primmer CR (2009) Unanticipated population structure of European grayling in its northern distribution: implications for conservation prioritization. ***Frontiers in Zoology***, 6, 6.

**II** Swatdipong A, Primmer CR and Vasemägi A. Historical and recent genetic bottlenecks in European grayling, *Thymallus thymallus*. **Accepted pending minor revision in *Conservation Genetic*s.**

**III** Swatdipong A, Vasemägi A, Niva T, Koljonen M-L, and Primmer CR High level of population genetic structuring in lake-run brown trout, *Salmo trutta*, of the Inari basin, northern Finland. **Submitted manuscript**.

**IV** Swatdipong A, Vasemägi A, Niva T, Koljonen M-L, and Primmer CR Mixed-stock analysis of lake-run brown trout populations to the fishery in the Inari basin, northern Finland: implications for conservation and management. **Manuscript.**

*"Conservation genetics is the application of genetics to preserve species as dynamic entities capable of coping with environmental change. It encompasses genetic management of small populations, resolution of taxonomic uncertainties, defining management units within species and the use of molecular genetic analyses in forensics and understanding species' biology."*

- Frankham et al. 2002 -

# 1. INTRODUCTION

## 1.1 Conservation biology

Conservation biology is a multidisciplinary field of science (figure 1) that aims to provide the principles, tools and knowledge for preserving the earth's biological diversity. It has been termed a 'crisis discipline' in a similar way to cancer biology (Soule 1985; Frankham et al. 2002) due to the fact that in many cases, conservation actions are initiated only after a species or population is already in extreme danger of going extinct.

## 1.2 Conservation genetics

In addition to ecosystem and species diversity, genetic diversity is the third fundamental level of biodiversity that the World Conservation Union (IUCN) has recognized as being important to preserve. This is because genetic diversity is fundamental to the future evolution and to the adaptive flexibility of a species is crucial for persistence in the inherently dynamic world. Therefore, the key to maintaining biodiversity and healthy ecosystems is to identify, monitor and maintain locally-adapted populations, along with their unique gene pools, upon which future adaptation depends. Thus, conservation genetics deals with the genetic factors that affect extinction risk and the genetic management regimes required to minimize the risk (Frankham et al. 2002).

The application of population and evolutionary genetic theory and methods to address issues of conservation relevance has a long history (reviewed by Frankel and Soule 1981; Schonewald et al. 1983). However, the formalization of conservation genetics as a research field is still relatively recent. For example, the journal *Conservation Genetics* was established in 2000 and the first text book devoted to conservation genetics was published in 2002 (Frankham et al. 2002), which has recently been followed by several others (Allendorf and Luikart 2006; Henry 2006; Moritz and Sherwin 2009). As such, conservation genetics can still be considered as a young discipline. Conservation genetics is an applied discipline that utilizes information from population, evolutionary and quantitative genetics to address issues of conservation relevance. Frankham et al. (2002) identified 11 major genetic issues in conservation biology (figure 2). Below, I outline how knowledge of genetics can aide conservation in those areas touched upon in my thesis.

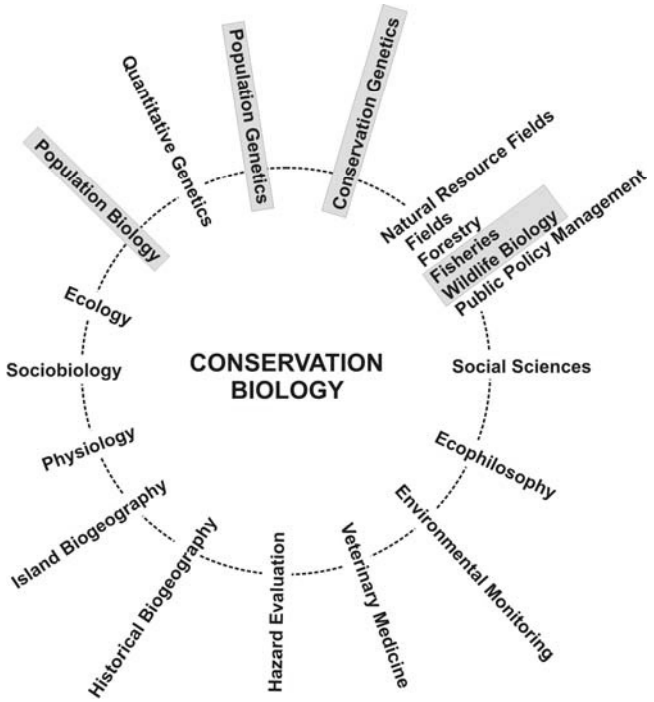


**Figure 1** Structure of conservation biology and its 15 related topics (modified from Soule 1985). Gray shaded topics are addressed in this thesis.

### 1.2.1 Small populations and the loss of genetic diversity

Small population size is a key theme in conservation genetics. This is because of the negative short and long term effects small population size has on the survival of populations. In the short term, small population size reduces survival and reproduction due to inbreeding depression (e.g. Saccheri et al. 1998; Hale and Briskie 2007). In the long term, small populations have a reduced capacity to respond to environmental changes (Frankham 1995). New genetic diversity in a species can be gained only by mutation that is rare and takes time, in the order of thousands of years. However, alleles and allelic combinations can be lost rapidly, even within a single generation. The great difference in the time period of gain/loss primarily motivates the conservation of genetic diversity within species. Therefore, the estimation of effective population size (the size of an idealised population that would experience the same magnitude of genetic drift as the population of interest; $N_e$), better understanding of the effects of small population size and the development and application of methods to identify cryptic population crashes are all key themes in conservation genetics. Estimation of effective population size and detection of population crashes were key themes in paper II and were also touched on in papers I and III.

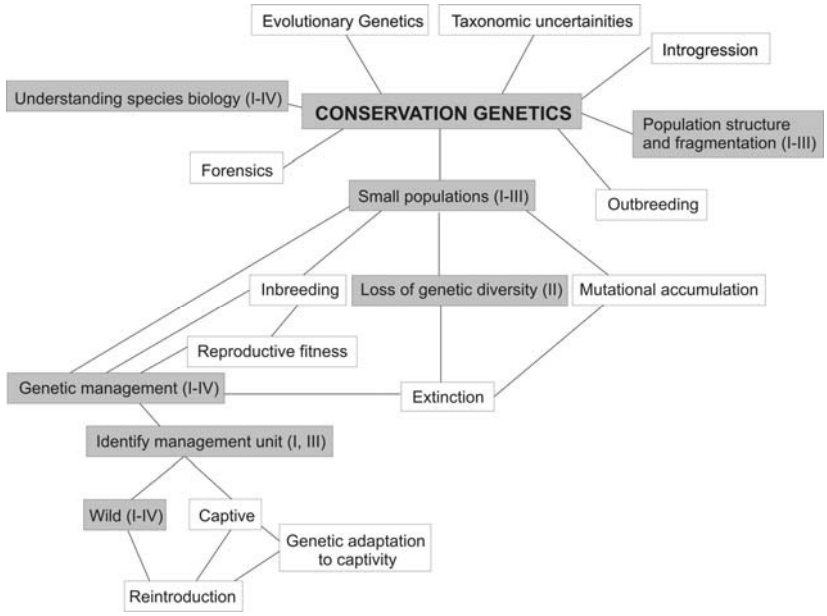


**Figure 2** Structure of conservation genetics (modified from Frankham et al. 2002). Gray shaded issues are thesis topics and Roman numerals refer to the chapters of my thesis in which the topics are addressed.

### 1.2.2 Population structure and fragmentation

Understanding the level of migration and the level of genetic differentiation among populations is very important for population management. Decreases in the level of migration due to human induced effects such as habitat loss and fragmentation are particularly important from a conservation perspective. This is because when gene flow is restricted, fragmentation often leads to greater inbreeding and loss of genetic diversity within fragments. In some cases, translocations or supplementation may be required to avoid the negative effects of inbreeding. For example, population differentiation should be considered when fish translocation is needed to enhance genetic diversity in small populations (Gross et al. 2001; Gum et al. 2006). In my thesis, the population structure of two salmonid fish species was studied in papers I and III.

### 1.2.3 Identification of management units

Natural populations within species may exhibit genetic differentiation to such a level that they should be managed as separate units. The justification for this is that the high level of differentiation may be due to the populations being adapted to different environments and/or the fact that there is limited gene flow between the populations. In salmonid fishes, high genetic differentiation among populations is common due to the high level of homing behavior. Salmonid fishes that are sedentary or completely resident can have very high levels of genetic differentiation among neighboring populations (Carlsson and Nilsson 2000; Koskinen et al. 2001). These natural populations are most likely adapted to their local habitat and inter-population hybrids might have low fitness. Therefore, such local populations should be recognized as separate management units (Small et al. 1998). In my thesis, the

identification of management units was studied in papers I and III.

### 1.2.4 Understanding species biology

A thorough understanding of the biology of a species is necessary for the development of any conservation plan. However, the biology of wildlife is often difficult to observe in practice. Several biological aspects can be revealed using genetic approaches. For example, fish migratory behavior and migratory patterns have been investigated using genetic analysis (Ruzzante et al. 2004; Ruzzante et al. 2006). This information can be used for sustainable exploitation. For example, harvest may be temporarily suspended during the running time of fish from small populations so that they are not threatened (Beacham et al. 2004). All of the papers in my thesis deal with understanding different aspects of the biology of salmonid fishes, including breeding site fidelity and feeding migration behavior.

## 1.3 Conservation genetics of exploited populations

When thinking about classic cases of conservation, one tends to think of socially appealing species such as birds or large mammals. Conservation efforts in such species are entirely focused on maximizing the survival of all individuals. In contrast, there is a key difference in aiming to promote the short and long term survival of exploited populations such as many fish species: at the same time as we want to preserve them, we want to eat them too! Clearly this results in a conflict and not just the biological conflict resulting from the processes of population decline and replenishment, but also social, and very often political, conflicts. A common source of conflict is disagreement about population size, both in terms of what minimum population size is necessary for sustainable harvesting as well as misunderstandings regarding the difference between census sizes and effective population size. The census size of exploited populations can often be large, especially in marine species (e.g. Poulsen et al. 2006). The extinction risk for large populations of exploited species is then not obvious based solely on population census size. However, it has been demonstrated that the loss of genetic diversity can still significant in large populations under a strong size reduction (Smith et al. 1991; Hutchinson et al. 2003). Therefore, a substantial harvest may have a high impact on any exploited population and a large population size does not necessarily mean ‘no risk’.

Partly because of these conflicts, exploited fishes, such as salmonids, have been threatened due to overfishing, intentional stocking, ocean ranching, spread of pathogens and parasites and escapees from aquaculture (Allendorf and Leary 1988; Hindar et al. 1991; Taylor 1991; Waples 1991; Leary et al. 1993). In order to compensate for the decline or extinction of many salmonid populations, large scale supportive breeding and release programs, also known as stock enhancement, have been initiated in many countries (Kitada et al., 2009). Supportive breeding programs usually use a fraction of the wild spawners for artificial reproduction, and their offspring are subsequently released into the natural habitat along with wild conspecifics. A key conservation aim of supportive release programs is usually to increase the number of spawners in natural stocks and simultaneously avoid the introduction of foreign genes into the overall naturally

reproducing population. There are, however, several potential drawbacks to the use of supportive releases as a conservation measure. For example, negative effects such as a decrease in genetic variability and/or effective population size as well as the replacement of wild populations by hatchery-born fish have been theoretically predicted (Ryman and Laikre 1991) or empirically observed (Taniguchi et al. 1983; Kitada and Kishino 2006; Kitada et al. 2009). In addition, even when local broodstocks are used for enhancement, genetic change can occur in the gene pool of local populations (e.g. Koskinen et al. 2002c).

Early fisheries genetics studies were based on blood group analysis (Cushing 1952; Cushing and Durall 1957). Later studies were based on isozymes (Hodgins et al. 1969; Aspinwal 1974), minisatellites (Galvin et al. 1995), microsatellites (McConnell et al. 1995) and major histocompatibility (MHC) loci (Beacham et al. 2005). The most recent marker of choice for many applications is the single nucleotide polymorphism (SNP) (Morin et al. 2004; Ryynanen and Primmer 2006). However, development of large numbers of SNPs can be time consuming, especially in species exhibiting low genetic diversity (Ryynanen et al. 2007). Hence the markers of choice throughout this thesis were highly variable microsatellite loci (see methods for more details).

### 1.4 The grayling and the brown trout

European grayling (*Thymallus thymallus*) and brown trout (*Salmo trutta*) are salmonid fishes i.e. belonging to the family Salmonidae, which includes Atlantic and Pacific salmon, charr, and whitefish in addition to trout and grayling. Many salmonids are anadromous, spawning in freshwater, then spending a number of years in the sea before returning to freshwater to reproduce. They are predators feeding on insects, benthos and small fishes. Salmonids are native to the northern hemisphere. In Finland, they are highly valuable for recreational (6105 tons, 15.6 million €, year 2006) and commercial fisheries (3859 tons, 8.7 million €, year 2007; www.rktl.fi). However, many salmonid populations have been negatively affected by human induced changes such as overfishing (Parrish et al. 1998), loss of habitat by construction of electro-power plants and weirs (Uiblein et al. 2001; Meldgaard et al. 2003), increased pollution (Arkoosh et al. 1998,), improper stock transfers (Gum et al. 2006) and infection by parasites and pathogens (OIE 2003) increasing the extinction risk of individual populations and creating challenges for sustainable fisheries management (WWF 2001).

The European grayling is native to Europe, with a distribution ranging from England and France to the Ural Mountains of north-western Russia. Unlike other salmonids, grayling tend to have sedentary behavior and complete their entire lifecycle in freshwater such as streams, rivers and lakes. Grayling also show large phenotypic variation in various meristic, morphological and life-history traits such as gill raker number, body size and weight at first spawning (Zinov'ev 2005). Many grayling populations are considerably threatened in several regions (Uiblein et al. 2001; Duftner et al. 2005) and grayling are listed as a protected species in appendix III of the Bern Convention. Natural grayling populations are common in northern Finland and can be also found in large lakes and streams in the eastern part of the country and have also been stocked in

other regions. Some populations occupy the brackish water environment in the Gulf of Bothnia. Recently, the status of 490 grayling stocks in Finland was evaluated and three distinct categories of populations were described (Kaukoranta et al. 2000): approximately 74% of Finnish grayling populations are classified as indigenous, 11% are mixed origin and the remaining 15% have been presumably introduced from Lake Ladoga, Russia. Of 253 Finnish populations which have been further evaluated in terms of conservation status, 16% are listed as declining or endangered (Kaukoranta et al. 2000). In addition, stock supplementation from hatcheries to support fisheries is a common practice in several parts of Finland today where grayling continues to be highly appreciated by recreational anglers as a game fish with annual catches of ca 300 tons (Finnish Game and Fisheries Research Institute, www.rktl.fi). Nowadays, 1.5 million one-summer-old grayling are released annually to natural resources (Finnish Game and Fisheries Research Institute, www.rktl.fi).

Phylogeographic studies have indicated that European grayling throughout Europe originate from four main mitochondrial lineages corresponding to different refugia during the last glacial period (Gum et al. 2009). Grayling populations in Finland belong to the lineage originating from the Russian refugium, near the Caspian basin (Koskinen et al. 2000), while European mainland populations originated from central European lineages and Norwegian and Swedish populations are in the contact zone of the Russian and central European lineages (Koskinen et al. 2000; Susnik et al. 2001; Gum et al. 2005). Grayling populations generally exhibit very low genetic diversity (Koskinen et al. 2002b; Gum et al. 2006) compared to other salmonids (Vasemägi et al. 2005; Gross et al. 2007; Fave and Turgeon 2008; Lehtonen et al. 2009) and are often genetically highly structured i.e. exhibiting high levels of genetic divergence, even among neighboring populations (Koskinen et al. 2001). Despite the quite extensive amount of genetic research, detailed national-level information of the population structure of European grayling in northern Europe is currently lacking. Yet this is the level at which populations are managed. Clearly, such information would be useful for efficient conservation and proper management of Finnish grayling populations as has been demonstrated for other salmonid species (e.g. Primmer et al. 1999; Østergaard et al. 2003; Fraser et al. 2004).

Brown trout is native to Europe, North Africa and western Asia (Elliott 1989). Currently, brown trout can be found worldwide as an introduced species and is popular for both professional and recreational fisheries (Youngson et al. 2003; Nylander 2004). Brown trout has a more flexible life history than its closest relative, Atlantic salmon (*Salmo salar*), ranging from completely stream resident to sea-run (anadromous) types, both of which can live sympatrically (Elliott 1994; Klemetsen et al. 2003). Trout spawn in freshwater and parr forage in their natal tributaries or rivers for on average 2-6 years before smoltification, largely depending on water temperature and food availability (L'Abee-Lund et al. 1989). Smolt subsequently migrates to a lake or sea to grow to maturity while the resident brown trout spends the entire life cycle in the natal stream. After maturation brown trout return to their natal site with high accuracy thus limiting gene flow which results in relatively high genetic differentiation among populations, even

between geographically close populations (Ryman 1983). In freshwater habitats, lake-run brown trout populations that migrate from streams to freshwater lakes to feed and return to streams to spawn appear to be equally susceptible to the negative effects of anthropogenic activities as sea-run migratory populations. An evaluation of the conservation status of Finnish lake-run brown trout populations indicated that 80% are classified as endangered (Kallio-Nyberg et al. 2001). Currently, more than half of the inland populations are supported by releases (Finnish Game and Fisheries Research Institute, www.rktl.fi). Population genetic research on brown trout has shown clear genetic structure and significant genetic differentiation among most of the populations occupying neighboring rivers and even tributaries within the same river (Carlsson and Nilsson 2000; Hansen et al. 2002; Fraser et al. 2007; Apostolidis et al. 2008). Population genetic studies in brown trout have concentrated on the within river level of either resident or sea-run populations (Carlsson et al. 1999; Charles et al. 2005; Antunes et al. 2006; Hovgaard et al. 2006) or on very broad regional scales (Bernatchez 2001; Presa et al. 2002; Schreiber and Diefenbach 2005). However, lake-run brown trout systems, the scale of which falls between within river and between regions, have been rarely studied (e.g. Duguid et al. 2006).

### 1.5 Objectives

The overall objective of my thesis was to apply and develop population genetic approaches to conduct a series of studies on exploited populations of grayling and brown trout in Finland in order to assist the conservation and sustainable harvest of these populations. These approaches included:

i) the characterization of the population genetic structure at national (I) and local (III) scales

ii) the identification of management units (I, III) and the prioritization of populations for conservation based on evolutionary forces shaping indigenous gene pools (I)

iii) the detection of population declines using genetic data and the testing of the assumptions underlying these tests (II)

iv) the evaluation of the contribution of natural populations to a mixed-stock fishery (IV) using information of the population structure (III)

# 2. MATERIAL AND METHODS

Nineteen European grayling populations were used to study population genetic structure within Finland (figure 3a). In addition, six populations from Russia, Sweden, Norway and Germany were used for comparative purposes between Finland and other countries. Finnish populations were categorized and prioritized based on levels of genetic differentiation and diversity in a conservation perspective aiming to retain total allelic richness at the national level (Petit et al. 1998; I). Another nine grayling populations from Russia, Denmark, Slovenia and Germany were included (II) to analyze recent and historical footprints of population bottlenecks. Complementary to the genetic bottleneck tests, the effective population sizes of grayling populations were estimated (II).

Twenty-eight brown trout populations were sampled from tributaries and rivers in the Inari basin, northern Finland (figure 3b), to obtain detailed knowledge about the genetic relationships among populations. As a particular focus, the level of genetic differentiation among populations was determined to identify management units for assisting conservation efforts. Additionally, old scale specimens (collected from 1949 to 1976) and spawners used in local hatcheries were compared with recent natural populations (mainly collected in 2008-2009) in order to characterize the temporal trends related to hatchery supplementation of wild populations (III). Mixed catches from Lake Inarijärvi and Lake Paadarjärvi were analyzed to determine the contribution of individual populations and population groups to the fisheries catch of the Inari basin (IV).

As noted above, microsatellite markers have been the markers of choice in fisheries and conservation genetics over the last 10-15 years. Microsatellites comprise simple sequence motifs of 1-6 nucleotides that are typically repeated between 5 to 40 times, i.e. $(AC)_5$, but longer tandem repeats are also found (Selkoe and Toonen 2006). Microsatellite loci exhibit high mutation rates, ranging from $10^{-2}$ to $10^{-6}$ per locus per generation (Ellegren 2000). In order to explain microsatellite variability patterns, several mutation mechanisms have been suggested; the infinite alleles model (IAM; Kimura and Crow 1964), stepwise mutation model (SMM; Ohta and Kimura 1973) and two-phase model (TPM; Di Rienzo et al. 1994). Additionally, null alleles, allele dropout and genotyping errors have to be carefully taken into account when using microsatellites as genetic markers (Pompanon et al. 2005). However, the great advantage of microsatellites, besides being highly variable, is that they are easily amplified and screened using gel or capillary electrophoresis and a large number of statistical tests and analyses methods exist for the analysis of microsatellite data (Excoffier and Heckel 2006). As a consequence, microsatellites are widely used in population and conservation genetics.

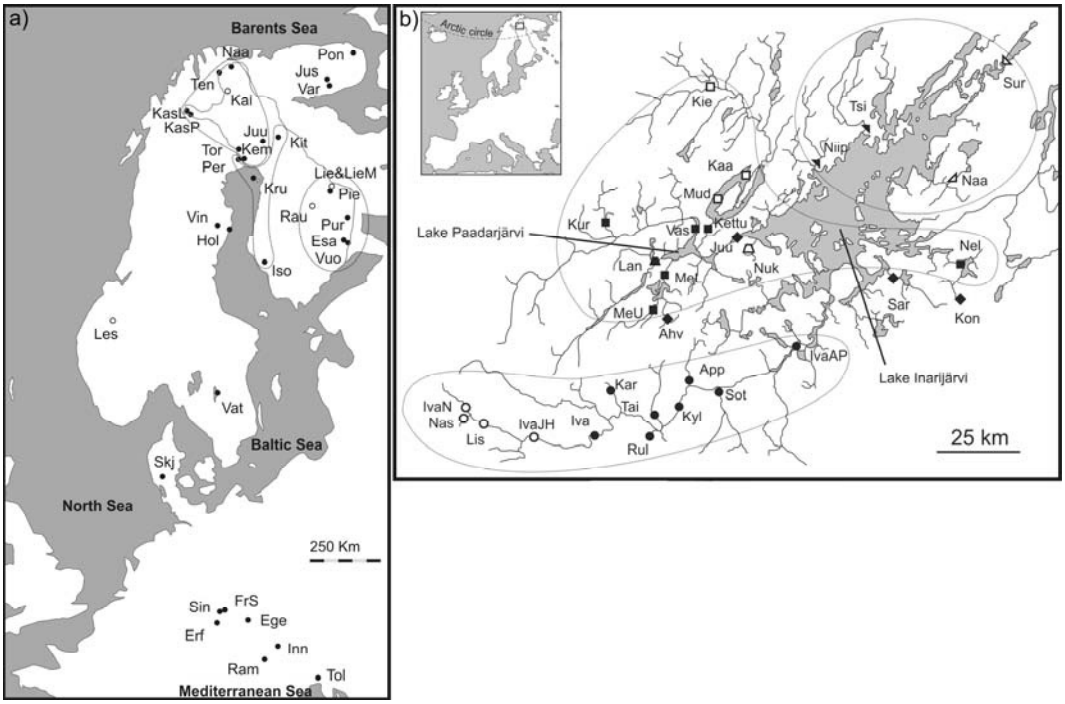


**Figure 3** Map indicating the locations of the studied salmonid populations. a) Three population clusters of European grayling, corresponding to northern, Baltic and south-eastern geographic areas of Finland, are indicated (I). All European grayling populations exhibited strong signs of historical genetic bottleneck (II) while four populations were also affected by more recent genetic bottleneck (open dots). b) Three population clusters of brown trout in the Inari basin are indicated (III) along with Lake Inarijärvi and Lake Paadajärvi where fishery catches were analyzed using mixed stock analysis (IV). The full names of populations are given in papers (I-IV). Note that three brown trout populations (Ahv, Kon and Sar) were sampled later and were thus not included in analyses in paper III.

For amplification of microsatellite loci multiplex PCR (the simultaneous amplification of >10 microsatellite loci) was used throughout the thesis. Optimization of the multiplex began by finding optimal annealing temperatures for individual microsatellite loci using gradient PCR. Typically, the temperature gradient ranged from 50-60°C and initial testing was done using equal concentration of primers (0.2 pmol/µl) as recommended in a commercial multiplex PCR protocol (Qiagen). The optimum annealing temperature was chosen based on unambiguous peaks for all loci in the amplicons. Subsequently, the multiplex PCR was further optimized using different concentrations of primers depending on the intensity of initial amplification. For example, the concentration of primers for loci with initial low peak heights was increased 2-3 fold. Loci that did not result in reliable amplification after several optimization rounds were excluded. Detailed multiplex PCR conditions and primer concentrations are described in papers I and III.

Characterization of population genetic structure represents one of the main themes of this thesis. Principle component analysis (PCA) on allele frequencies was primarily

used to visualize the population structure on both national and local scales (I, II). To obtain more detailed information on the population structure, a Bayesian clustering algorithm which utilizes the information of individual multilocus-genotypes (Structure 2.2; Pritchard et al. 2000) was employed in paper I, while the information about groups of individuals was taken into account when inferring the local population structure in paper III (Baps 5.2; Corander et al. 2004). Analysis of molecular variance (AMOVA; Arlequin 3.11; Excoffier et al. 2005) was used to evaluate the amount of variation associated with a particular population hierarchy (I, III). Population genetic simulations were routinely used to test population genetic models and their underlying assumptions (II, IV). The effect of migration on the dynamics of the *M* ratio (Garza and Williamson 2001) was re-evaluated (II) using simulated data (Easypop 1.8; Balloux 2001) under various migration rates and time scales. The accuracy of mixed stock analyses (Oncor, www.montana.edu/kalinowski/Software) was evaluated using simulated mixtures of fish with known-origin (IV; PopTools 3.0; Hood 2008).

All laboratory procedures and genetic data analyses are detailed in the original papers.

# 3. RESULTS AND DISCUSSION

**Paper I** This study aimed to reveal the national-scale population structure of Finnish grayling and to prioritize the conservation of natural populations according to genetic differentiation and diversity. Regional population structure was found in Finnish grayling that corresponds to three geographic regions of Finland (northern, Baltic and south-eastern; figure 3a). The regional clustering was evident using both PCA and individual multi-locus genotype based analysis (figure 4) and further supported by analysis of molecular variance. The allele permutation test indicated that the relatively high differentiation among the three population groups was mostly driven by genetic drift and low migration rather than accumulation of new mutations. PCA and individual clustering strongly indicated that the Kitkajärvi population groups together with the Baltic populations even though this population inhabits an inland lake that is relatively distant (174 km) from the Baltic Sea and, moreover, currently flows into the White Sea basin. It was hypothesized that the current Kitkajärvi grayling population was established more than 8 400 years ago through the historical waterway from the Baltic Sea (Heikkinen and Kurimo 1977).

In this paper, a novel population categorization method was developed, based on the relative role of neutral evolutionary forces (mainly drift and migration) along the genetic diversity and differentiation axes (figure 5). Four population categories were described; 1) high diversity-high differentiation, 2) high diversity-low differentiation, 3) low diversity-high differentiation, and 4) low diversity-low differentiation. Populations in category 1 are suggested to exhibit relatively large population size and highly diverged from other populations. Thus, populations in category 1 have the highest likelihood of containing unique genetic material and the top priority for conservation, while those in categories 2-4 have lower priority.

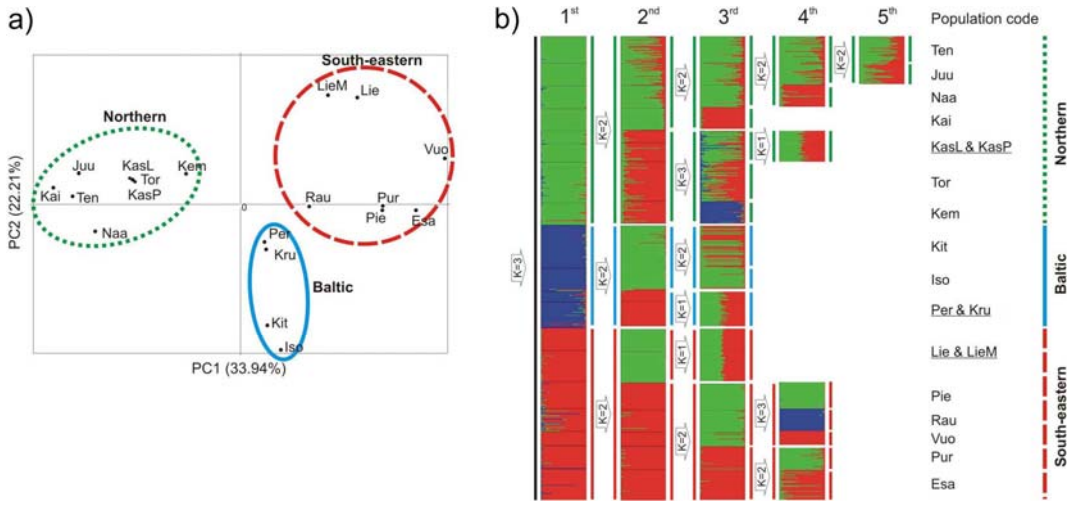


**Figure 4** Clustering of Finnish grayling populations based on 13 microsatellite loci. Population codes are described in table 1 of paper I. a) Principal component analysis of Finnish grayling populations based on allele frequencies. b) Individual clustering as inferred by a series of hierarchical partitioning using Structure. Each individual is represented by a thin horizontal line pooled into K-colored blocks indicating an individual's membership fractioned in K clusters. Black horizontal lines separate individuals from different sampling sites.

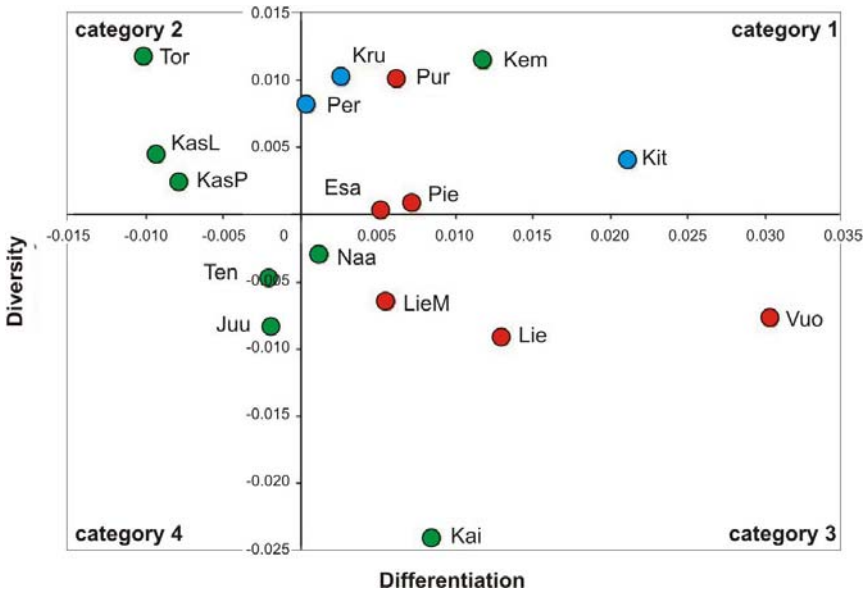


**Figure 5** Population prioritization of wild Finnish grayling populations along the genetic diversity and differentiation axes. Four different prioritization categories were identified: high diversity-high differentiation (Category 1), high diversity-low differentiation (Category 2), low diversity-high differentiation (Category 3) and low diversity-low differentiation (Category 4). Populations are colored differently according to the PCA separation of the three population clusters and are coded as in table 1 of paper I.

**Paper II** This study combined information from paper I with published data (Koskinen et al. 2002b; Gum et al. 2003; Korkea-Aho 2003) to further analyze European grayling populations covering the majority of the species' distribution range. A strong global footprint of population size reduction in all analyzed populations (n=34) was detected based on *M* ratio test. Using a coalescent approach the beginning of the population decline was dated back to 1000-10000 years ago (ca. 200-2000 generations; figure 6) and current populations were estimated to represent only 0.03-1.2% of historical sizes. Forward simulations demonstrated that the bottleneck footprints measured using the *M* ratio can persist within small populations much longer than previously anticipated in the face of low levels of gene flow (figure 7). Specifically, the time window when the genetic footprint as manifested as a low *M* ratio is detectable was re-evaluated and proved to be much longer (up to 3000 generations) than originally determined by Garza and Williamson (ca. 350 generations; Garza and Williamson 2001). This clearly emphasizes the importance of gene flow in compensating for the effects of genetic drift and loss of variation over long evolutionary time scales. The bottleneck simulation also suggests that the low *M* ratio is unlikely to be frequently observed in species with high gene flow levels, even following a dramatic reduction in population size. In addition to the low *M* ratio, more recent bottleneck footprints were identified in six populations using alternative bottleneck tests using the Wilcoxon's sign rank test on heterozygosity excess and the mode-shift test (Cornuet and Luikart 1996; Luikart et al. 1998). From the conservation perspective, careful monitoring of these populations is warranted. Consistent with the predominant role of random genetic drift, the effective population size ($N_e$) estimates of all grayling populations were very low with the majority of $N_e$ estimates below 50.

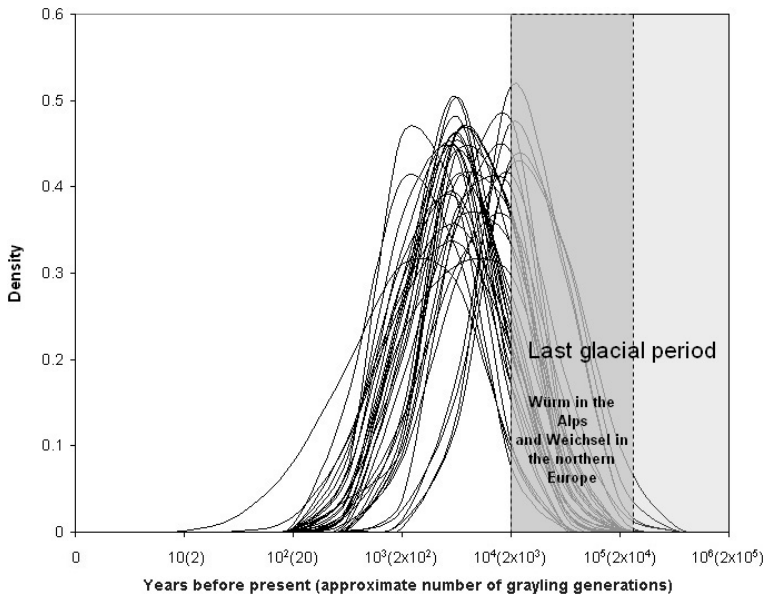


**Figure 6** Estimated time since the start of population decline (posterior distribution) in 34 grayling populations across Europe using an exponential model in the program MSVAR (Beaumont 2004). The gray shaded areas correspond to the last glacial period and its last sub-period (Würm and Weichsel period).

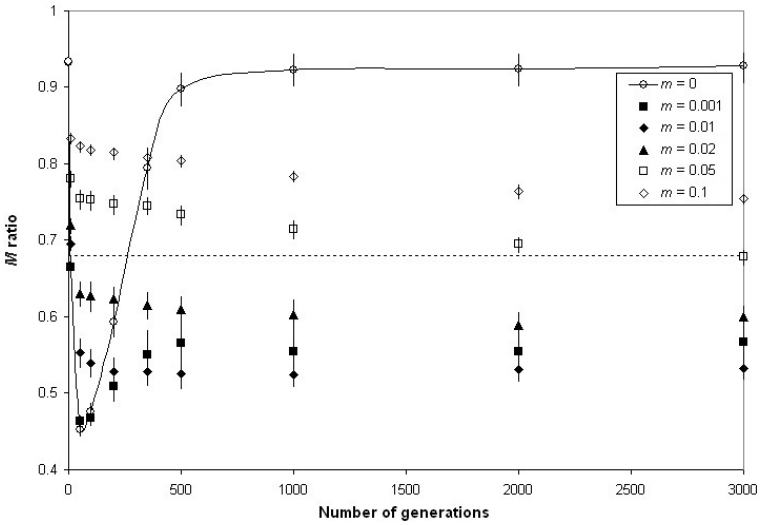


**Figure 7** The dynamics of the *M* ratio (means and 95% confident intervals) in the presence of migration (*m*) during the permanent bottleneck scenario. The uppermost circle on the left-side of the figure corresponds to the initial *M* ratio before the population reduction from 10000 to 50. A broken horizontal line represents the critical bottleneck threshold at 0.68, indicating past population bottleneck according to Garza and Williamson (2001).

**Paper III** This study aimed to characterize population genetic structure of lake-run brown trout in the Inari basin, a large lake system in northern Finland. Twenty-eight brown trout populations were clustered into three separate genetic groups largely corresponding to geographic regions (northern, east-western and southern of the basin; figure 3b, figure 8). Genetic differentiation among the northern, east-western, southern population groups was particularly high ($F_{ST}$=0.11-0.16). Significant differentiation was also observed even among populations from tributaries in the same river system. Trout from populations in the Juutunajoki river system were suggested to feed in small lakes in the system rather than fully rely on Lake Inarijärvi, the main lake of the basin. Lake-run brown trout populations in the Inari basin exhibited a significant isolation-by-distance signal ($R_{XY}$=0.496, P=0.003) and a tendency for genetic diversity to decrease with increasing distance from the river mouth (Pearson's correlation coefficient r=0.846 for allelic richness and r=0.805 for expected heterozygosity, P<0.001 in both cases). By comparing to other systems, lake-run trout in the Inari basin had a level of structuring similar to that of the sea-run trout, but clearly lower than the resident trout. However, the correlation between genetic and riverine distances in all brown trout systems was much steeper than that of Atlantic salmon, indicating a higher level of structuring in brown trout (figure 9).

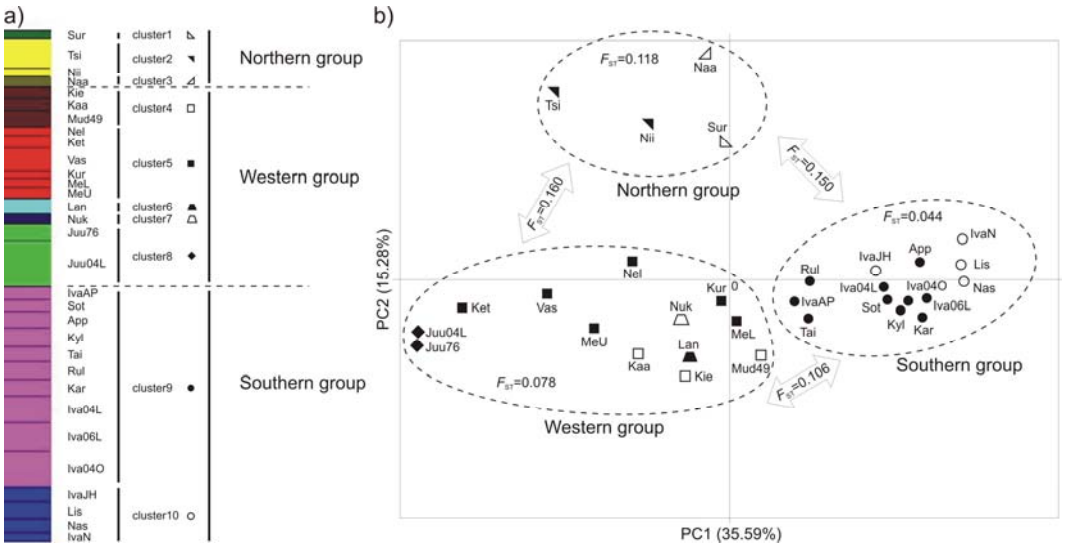


**Figure 8** Population structure of Inari brown trout identified as three genetic groups based on: a) Bayesian clustering analysis (Baps); b). Principle component analysis. In (a), each population is represented by a square block with the size corresponding to the number of individuals within the population. Both color and symbol indicate a cluster membership. $F_{ST}$ measurements within (in the oval) and among groups (in the 2-headed arrow) are also indicated in (a). Populations are coded as in table 1 of paper III.

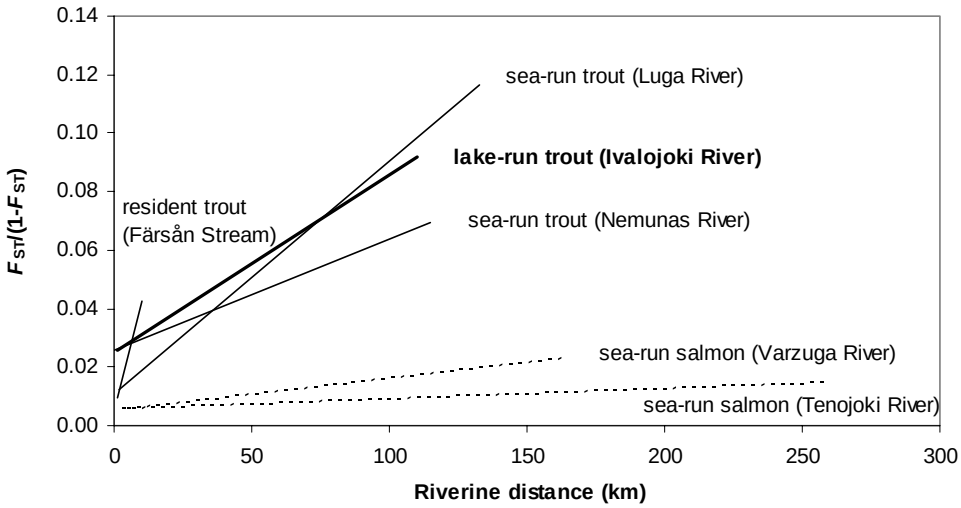


**Figure 9** Isolation-by-distance trends in brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) using linear regression lines (continuous and broken lines, respectively). The linear regression line observed in paper III is marked in bold.

**Paper IV** This study aimed to estimate population contribution in mixed stock fishery samples of wild lake-run brown trout to assist fishery management in the Inari basin, northern Finland. Twenty-eight populations from paper III and three additional populations were used as references for mixed stock analyses on catches of natural fish from Lake Paadarjärvi and Lake Inarijärvi (figure 3b). Altogether twelve populations contributed significantly to mixed stock fisheries (figure 10b) with the Ivalojoki river system being the major contributor (70%) to the total catch, following by the population group from the Juutuanjoki river system (15%; figure 10a). The high proportion of trout from the Ivalojoki river system (southern population group) in the total catch from Lake Inarijärvi was initially unexpected given the high number of rivers and streams that flow into the lake. However, when evaluating the habitats available for reproduction and juvenile feeding it is evident that the suitable areas of the Ivalojoki river system are far larger than in the other rivers (>400 ha vs. ~100 ha or less).

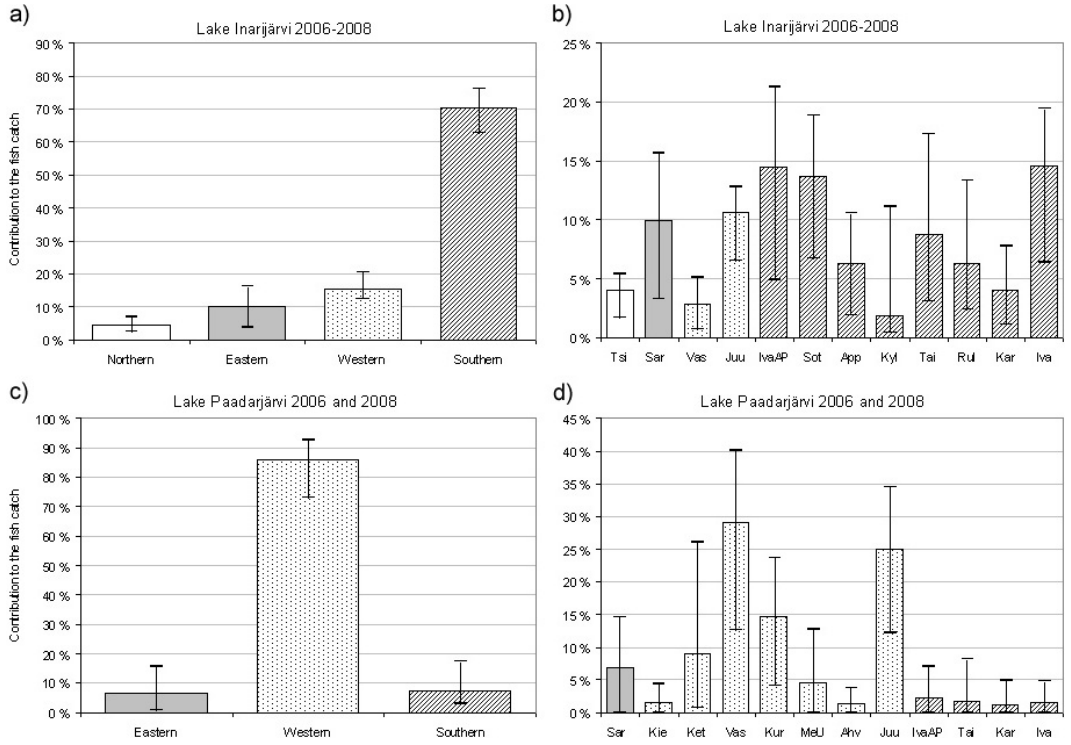


**Figure 10** Contribution of each brown trout group and population to catches from the Inari basin: Lake Inarijärvi (n=523), estimates of group (a) and population (b), Lake Paadarjärvi (n=139), estimates of group (c) and population (d). Group or populations that contribute <1% of fish catches are not shown. Note that the eastern and western groups are in the same PCA cluster.

When the contribution of wild trout populations to the fisheries was evaluated regionally, geographically nearby populations were the main contributors to the local catches. This indicates that a considerable number of trout occupy lacustrine feeding grounds nearby the natal river mouth, rather than dispersing through Lake Inarijärvi. The brown trout catch from Lake Paadarjärvi was dominated by populations from the western group (86%; figure 10c) which consists of nearby populations from the Juutuanjoki river system (Vas, Juu, Kur; figure 3b, figure 10d) indicating that the contribution from geographically close trout populations is of primary importance for local fisheries also in Lake Paadarjärvi. MSA also revealed a clear separation between the lower and upper reaches of the Ivalojoki river system – in contrast to the lower reaches of the Ivalojoki river that contributed considerably to the catch, populations from the upper reaches of the Ivalojoki river (>140 km from the river mouth) did not contribute significantly to the fishery. However, the different level of contribution could also be at least partially related to the available habitat size. Comparative analyses of growth patterns of fish from the upper and lower reaches of the river would be necessary to obtain further evidence about the potential life history differences and trade-offs within the river system.

# 4. CONCLUSIONS AND FUTURE DIRECTIONS

## 4.1 Conclusions

Based on microsatellite analyses, clear genetic structuring of exploited Finnish grayling and brown trout populations was detected at both national and local scales. Finnish grayling were clustered into three genetically distinct groups, corresponding to northern, Baltic and south-eastern geographic areas of Finland. Genetic differentiation among and within population groups of grayling ranged from moderate to high. Such strong genetic structuring combined with low genetic diversity strongly indicates that genetic drift plays a major role in the evolution of grayling populations. Further analyses of European grayling covering the majority of the species' distribution range indicated a strong global footprint of population decline. Using a coalescent approach the beginning of population reduction was dated back to 1 000-10 000 years ago (ca. 200-2 000 generations). Forward simulations demonstrated that the bottleneck footprints measured using the *M* ratio can persist within small populations much longer than previously anticipated in the face of low levels of gene flow. In contrast to the *M* ratio, two alternative methods for genetic bottleneck detection identified recent bottlenecks in six grayling populations that warrant future monitoring. Consistent with the predominant role of random genetic drift, the effective population size ($N_e$) estimates of all grayling populations were very low with the majority of $N_e$ estimates below 50. Taken together, highly structured local populations, limited gene flow and the small $N_e$ of grayling populations indicates that grayling populations are vulnerable to overexploitation and, hence, monitoring and careful management using precautionary principles is required not only in Finland but throughout Europe.

Population genetic analyses of lake-run brown trout populations in the Inari basin revealed hierarchical population structure where individual populations were clustered into three population groups largely corresponding to different geographic regions of the basin. Similar to my earlier work with European grayling, the genetic differentiation among and within population groups of lake-run brown trout was relatively high. Such strong differentiation indicated that the power to determine the relative contribution of populations in the mixed fisheries should be relatively high. Consistent with these expectations, high accuracy and precision in mixed stock analysis (MSA) simulations were observed. Application of MSA to indigenous fish caught in the Inari basin identified altogether twelve populations that contributed significantly to mixed stock fisheries with the Ivalojoki river system being the major contributor (70%) to the total catch. When the contribution of wild trout populations to the fisheries was evaluated regionally, geographically nearby populations were the main contributors to the local catches. MSA also revealed a clear separation between the lower and upper reaches of Ivalojoki river system – in contrast to lower reaches of the Ivalojoki river that contributed considerably to the catch, populations from the upper reaches of the Ivalojoki river system (>140 km from the river mouth) did not contribute significantly to the fishery. This could be related to the

available habitat size but also associated with the resident type life history and increased cost of migration. These results have important implications for conservation and sustainable fisheries management of the Inari basin.

The studies in the thesis highlight the importance of dense sampling and wide population coverage at the scale being studied. The clear population structure at the national scale in paper I was revealed due to analyses of the larger number of populations compared to previous studies (e.g. Koskinen et al. 2000; Koskinen et al. 2002b). The importance of wide population coverage was further highlighted in paper II where European grayling populations from the majority of the species distribution range were analyzed and identified a strong global footprint of population bottleneck. As a consequence, if the bottleneck analyses would have originated from a small number of populations, our interpretation of patterns of reduced variability would have been different. The high level of precision observed in mixed stock analysis in paper IV further indicates the importance of adequate size of reference populations. In addition, papers I-IV demonstrate the potential and utility of combining different datasets for common analyses.

Computer simulations also played a significant role in this thesis. Simulations were routinely used to test population genetic models and their underlying assumptions. In paper II, the genetic bottleneck simulations were extended to evaluate the effect of migration on the dynamics of the *M* ratio test. These results strongly indicated that a low *M* ratio, commonly believed to represent a signal of rather recent genetic bottleneck (maximally few hundred generations), can persist within small populations much longer than previously anticipated in the face of low levels of gene flow. Hence, based on coalescent and forward population genetic simulations, the low *M* ratio in European grayling is more consistent with the common historical genetic bottleneck scenario related to the last glaciation and subsequent post-glacial colonization assuming that low to medium levels of migration occur between populations. In the same study, a newly developed effective population size ($N_e$) estimator (Koyuk et al. 2008; Tallmon et al. 2008) was also tested and demonstrated that low levels of migration did not noticeably bias the $N_e$ estimates. The computer simulations had also a substantial role in evaluating the reliability of mixed stock analyses in paper IV. Taken together, this thesis demonstrates the importance of critical evaluation of the underlying assumptions of the population genetic models and methods used. As such, population genetic simulations, both forward and reverse in time, represent an important and efficient means for such work.

### 4.2 Future directions

This PhD thesis contains several questions and research themes that can be developed further. For example, it is important to remember that grayling and trout populations that were analyzed in this thesis represent only a small proportion of the populations that inhabit different rivers and lakes in Finland. Hence, there is still a considerable gap between the number of analyzed samples and the number of populations present in nature. Such discrepancy between the sampling coverage and the number of actual

populations can have important implications for genetic analyses. For example, as highlighted in paper II, if genetic analyses are conducted on samples collected from a single watershed location, as is often the case, the $N_e$ estimates can reflect effective population size of the local sub-population rather than that of the overall water system. Hence, studies examining even larger number of grayling populations from the central to northern part of Finland as well as testing the temporal stability of allele frequencies are warranted in order to shed more light on this issue. To that end, there are still a number of gaps to fill using additional samples to enable more comprehensive overview about the relative role of different evolutionary forces that can facilitate more effective conservation and management practices. The developed methods such as the 13-locus multiplex PCR and microsatellite panel described in paper I could assist towards reaching such goals by reducing future laboratory work. This PhD thesis also provides a solid framework for future mixed stock analyses efforts in the Inari basin as almost all brown trout populations in the basin were sampled and the reference dataset is ready for future study of the migratory behavior and feeding preferences of the lake-run trout populations. Similar to grayling, the genetic screening methods developed, such as the 13-locus multiplex PCR and microsatellite panel for brown trout, could help with fast and effective genotyping in the future. Therefore, the molecular tools and the reference dataset generated in my thesis will contribute to conservation and sustainable fisheries management of both lake-run and resident trout populations of the Inari system in the future.

Throughout this thesis I analyzed neutral molecular markers (microsatellites) that presumably are not affected by natural selection and most of the inferences in the thesis are based on the relative roles of neutral evolutionary forces such as genetic drift, migration and mutation. As a result, the potential role of natural selection on natural populations of European grayling and brown trout is only indirectly discussed and not directly evaluated. Hence, studies assessing genes and genomic regions potentially affected by natural selection would be interesting to conduct in the future. Further, given the high level of inter-population genetic differentiation observed in both grayling and brown trout, studies aimed at further investigating the relative importance of genetic drift and natural selection in explaining the observed genetic differentiation are recommended (e.g. Hansen et al. 2002; Vähä et al. 2008). In addition, given that salmonid populations are suggested to commonly evolve local adaptations (Garcia de Leaniz et al. 2007), studies aimed at testing the local adaptation paradigm would be particularly interesting. These could include e.g. reciprocal transplantations of local populations (Larsen et al. 2008) and common garden experiments in order to contrast quantitative genetic differentiation ($Q_{ST}$) and neutral molecular differentiation ($F_{ST}$) among different populations (Koskinen et al. 2002a; Jensen et al. 2008). Alternatively, genes and genomic regions under directional natural selection can be identified using hitchhiking mapping approaches (Schlotterer 2003; Vasemägi et al. 2005; Makinen et al. 2008) and the candidate genes and regions subsequently focally studied to find the actual targets of selection (Hemmer-Hansen et al. 2007; Andersen et al. 2009). Such studies would

complement the work conducted in this thesis on assumedly neutral genetic variation and provide additional valuable knowledge regarding adaptive variation which could be used to further fine-tune management strategies.

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